Chapter 1
The Potential of Castor as a Biodiesel Feedstock Crop for the Arabian Peninsula

Kiran Menon, S. Irshad Ahmed, Neeru Sood and Nanduri K. Rao

Abstract There is an increasing need to turn to biofuels to meet growing world fuel requirements. Oil-rich regions such as the Arabian Peninsula should be no exception in exploring alternative, renewable and environment-friendly fuel options in order to decrease their dependency on non-renewable fossil fuels. In this study we have carried out field trials of castor (*Ricinus communis*) in order to assess its suitability as a biodiesel feedstock crop in the region. We have also studied the response of 11 hybrid accessions of castor to three saline irrigation water treatments (5, 10 and 15 dS/m) and determined that castor can tolerate up to 5 dS/m salinity in irrigation water without any negative effect on oilseed yield.

Keywords Castor · Biodiesel feedstock · Salinity tolerance

1.1 Introduction

Castor (*Ricinus communis* L.) is a member of the Euphorbiaceae family. It is a perennial plant growing to a height of 2–3 m. The fruit is a globose capsule, 2.5 cm in diameter, usually containing three seeds. Yields of up to 5,000 kg/ha have been reported under irrigated conditions, but they can be less without adequate moisture [1]. In India, castor is cultivated in marginal lands and under rainfed conditions and so productivity is limited to around 1,200 kg/ha. In the wild, castor is able to adapt to arid conditions and withstand long periods of drought [2]. Castor seeds contain up to 60 % oil which is inedible. This makes it an ideal candidate for bio-diesel

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production due to the absence of competition with food crops. Bio-diesel from castor oil has properties such as very low cloud point and pour point which make it suitable for use in extreme winter temperatures. A single reaction step is required for the trans-esterification process of castor oil because of its favorable acidity level. Therefore, in a large-scale process, it would be less costly to produce bio-diesel from castor seeds than with others with a higher acidity level. The properties of the B100 (100 % biodiesel) combustible and its B10 and B20 blends (10 % and 20 % bio-diesel blends in petroleum diesel respectively) are comparable to those of petroleum diesel and acceptable within international bio-diesel standards (ASTM D 6751) with the exception of viscosity and humidity of B100 [2].

The countries in the Arabian Peninsula are at present entirely dependent on fossil fuels to meet increasing energy requirements. Oil and gas reserves will be depleted in the next 30–35 years [3]. According to the World Resources Institute, countries of this region such as Kuwait and UAE have some of the highest greenhouse gas emissions per capita as of the year 2010 [4]. Due to these reasons, the local governments have been taking serious initiatives for the reduction of carbon footprint and investing in alternative fuels.

There is also presently great interest worldwide in the use of marginal lands for the production of biodiesel feedstock crops because all arable lands need to be employed in meeting world food requirements. More than 6 % of the world's land area is affected by salinity [5]. Salts accumulate in soil over a period of time due to the weathering of parental rocks, which releases soluble salts of various types, mainly chlorides of sodium, calcium, and magnesium, and to a lesser extent, sulfates and carbonates [6]. Salinity is of increasing concern in the region due to overexploitation of groundwater, which is leading to a disturbance in the balance between sea and groundwater and deterioration of groundwater quality [7]. There have been reports on the salinity tolerance of castor, with some varieties being touted as more tolerant to salinity and sodicity than others [8, 9]. A few other studies however report that castor is sensitive to salt stress and that this may be due to a lack of efficient activity of guaiacol peroxidase and catalase enzymes, which probably leads to imperfect H_2O_2 scavenging [10]. Salinity levels above 10 dS/m have also been seen to affect the germination of castor seeds [11]. In this study we report the results of a field trial to assess the salinity tolerance and the suitability of UAE's growing environment for the large-scale production of castor oilseeds.

1.2 Materials and Methods

1.2.1 Field Trials

Eleven hybrid accessions of castor obtained from Vibha Seeds™, Hyderabad, India were used in this study, conducted at ICBA research station (25.09°N, 55.38°E) during the cropping season 2012–2013. The soils of the experimental site were sandy with very low organic matter, hence, farm yard manure was added at the rate of 40 tons/ha to improve the fertility. A Completely Randomized Block Design (CRBD) with three replications was used to evaluate the performance of the eleven hybrids. The seeds were sown in field plots, each of 4 rows of 3 m, with a distance of 50 cm both between the rows as well as between the plants within the row. Three salinity treatments with electrical conductivities (ECw) of 5, 10, 15 dS/m were established by mixing saline ground water (22–25 dS/m) with sweet water (3–0.5 dS/m), in addition to the control treatment, irrigated with low quality municipal water having an electrical conductivity of 0.3–0.5 dS/m. The plants were irrigated twice daily by drip irrigation at the flow-rate of 4 l/h per dripper.

From each plot, five plants were selected randomly and tagged for recording observations, which included qualitative traits such as growth habit, stem color, leaf color, spike type, spike compactness, waxy coating, fruit surface and fruit dehiscence, and quantitative traits such as plant height, number of branches, stem diameter, leaf surface area, leaf dry weight, leaf specific weight and moisture content, leaf chlorophyll content, spike length, number of spikes and fruits per plant, fruit size, seed yield and 1,000 seed weight.

1.2.2 Leaf Measurements

The leaf at the node of the primary inflorescence was chosen as a standard for all leaf measurements. Leaves from five plants in each plot were harvested at maturity and images were captured against a marked scale. In all instances, the surface area was determined using the ImageJ Image processing and the analysis software provided as freeware by the National Institutes of Health (NIH), US, which is a benchmark tool for image analysis and area measurements [12–14]. Leaf dry weight was recorded after the collected leaves (five from each plot) were washed in tap water to remove dirt and dried in a ventilated oven at 80 °C for up to 96 h until all the moisture had been removed and a static weight was reached. Leaf punches of 4 cm^2 surface area were weighed for specific weight values. The punched sections were then dried at 60 °C till a static weight was attained to obtain the dry weight. These values were then used to determine standard leaf weight (SLW) and moisture content (%) [15]. Leaf chlorophyll content (a, b and total) was estimated using the Mackinney method that was described in 1941 [16], improved by Arnon in 1949 [17], which is still the most widely used method for simple chlorophyll quantification [18].

1.2.3 Soil and Water

Five soil samples each were collected from the root zone of all four treatment plots. The pH of saturated soil paste was recorded using a pH meter. The salinity readings were recorded using a Conductivity Meter. Irrigation water samples were collected from each treatment to measure the pH and EC values.

1.2.4 Seed Oil Content

Oil was extracted using n-Hexane for solvent extraction using a Soxhelet apparatus. Oil weight was measured periodically and extraction was stopped when static weight was obtained.

1.2.5 Leaf K^+/Na^+ Ratio

Castor leaves (from the node of the primary inflorescence, 5 samples per plot), were dried at 80 °C till a static weight was observed (to remove all moisture). Dried leaves were ground using mortar and pestle and weighed samples were wet digested using concentrated HNO3 for 48 h. Leaf extract was filtered using ashless filter paper and analyzed using Inductively coupled plasma/optical emission spectrometry (ICP-OES). K^+ and Na^+ concentrations in leaf extracts were determined by ICP OES and K^+/Na^+ ratios calculated.

1.2.6 Ion Concentrations in Irrigation Water

Na and K ion concentrations (average of 5 samples for each treatment) in irrigation water were determined by flame photometry.

1.2.7 Statistical Analysis

Analysis of variance (two-factor ANOVA) was used to identify the traits that differed significantly among the accessions and those significantly affected by salinity, with the limit for statistical significance set at $p = 0.05$. Seed yield is expressed in kilograms per hectare based on extrapolation of the average yield of the five plants from each plot and assuming a stand of 36,000 plants per hectare, at the same density in the current field trial.

1.3 Results and Discussion

The results show that qualitative traits such as leaf and stem color and growth habit were not affected by salinity and stayed the same for any given accession across the different saline treatments. In contrast, quantitative traits were significantly affected when irrigated with saline water as revealed by the analysis of variance.

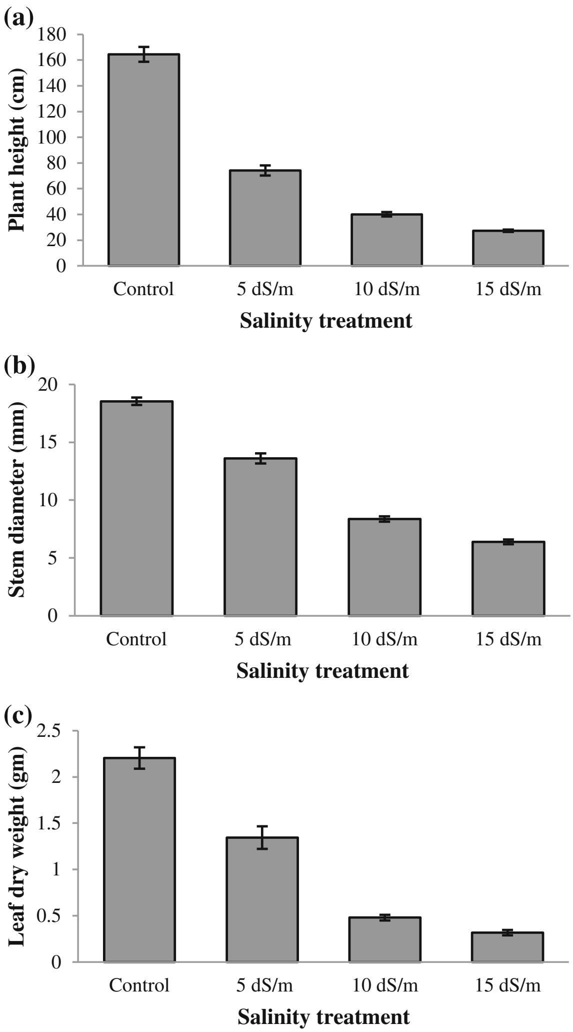

Fig. 1.1 Decrease in plant height (**a**), stem diameter (**b**) and leaf dry weight (**c**), with increase in salinity. Variance of data is denoted by standard error bars

Thus, compared with the control, plant height, stem diameter and leaf dry weight have all decreased considerably when irrigated with saline water (Fig. 1.1). As with many other abiotic stresses, plant growth is inhibited by salinity. A possible reason is stomatal closure resulting in decreased uptake of carbon dioxide and reduced photosynthesis or inhibition of cell growth and division [19]. The direct effects of salinity stress on cell expansion and division are not yet fully understood. In our study however, the rapid and extreme response of the plants in the 10 and 15 dS/m treatments suggests that the osmotic stress at such high salinity induces a stress response and stalling of growth in these plants. This is because high salinity in the root area decreases the plants capability to absorb water (osmotic stress) [20]. Within each treatment, the differences among the accessions were found to be

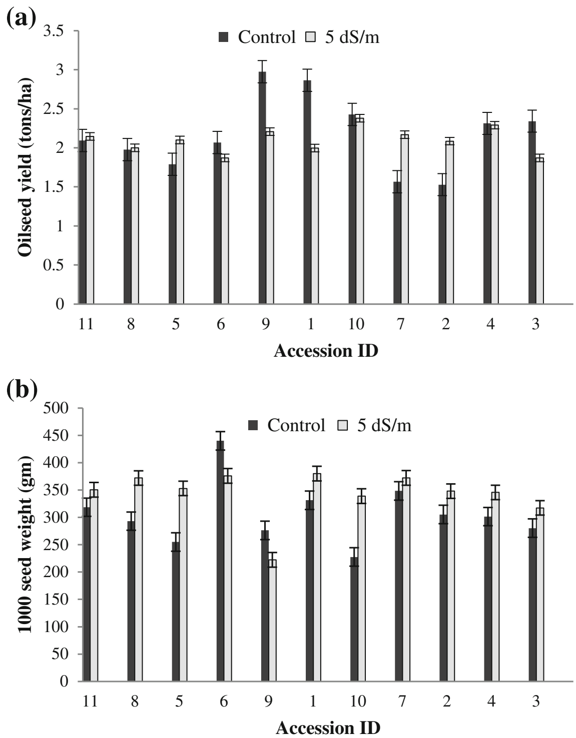

Fig. 1.2 Oilseed yield (**a**) and 1,000 seed weight (**b**) values for control and 5 dS/m treatments

insignificant. Analysis of variance also showed significant effect of salinity on leaf surface area, primary inflorescence length and leaf moisture content (data not presented). The reduction in leaf are is expected, as this reduces the amount of water used up by the plant. Compared to the control, leaf chlorophyll content increased in the 5 dS/m treatment and then decreased in the 10 and 15 dS/m treatments. The reasons for this were not clear, but further analysis needs to be carried out to confirm these findings.

With regard to seed yield and 1,000 seed weight, the results show that castor could tolerate salinity of up to 5 dS/m without a statistically significant decrease (Fig. 1.2). ANOVA results show that the difference in yield is not statistically significant between the freshwater and 5 dS/m treatments. The differences in yield of individual accessions (a decrease of yield in accession nos. 9 and 1 but increase in 7 and 2) can be attributed to genetic variation and associated differences in response to stress, but these differences are not of statistical significance. In the 5 dS/m salinity treatment, the seed yields were between 1.8 and 2.3 tons/ha for different accessions while freshwater cultivation yielded between 1.5 and 3 tons/ha of seed. In comparison with the freshwater treatment, seed yield decreased by 66 and 82 % with increase in salinity to 10 and 15 dS/m, respectively. The fact that seed yield is not significantly affected by salinity in the 5 dS/m treatment suggests that the possible ionic stress faced by the plant which results in decreased plant

Table 1.1 Salinity measurements for soil and irrigation water

Treatment	Salinity eC (dS/m)	
	Soil	Water
Control	1.714	0.339
5 dS/m	4.9542	4.68
10 dS/m	12.246	9.16
15 dS/m	18.586	16.06

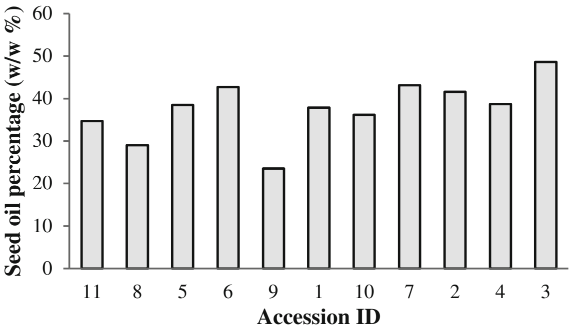

Fig. 1.3 Seed oil content in control treatment (w/w %)

height is not very damaging. Sodium ions may accumulate to an extent enough to reduce the plants' metabolic activity and growth, but not to a toxic level. This result is key because seed yield is the most important parameter in terms of commercial viability of cultivation. Within each treatment, differences between accessions for seed yield and 1,000 seed weight were found to be insignificant.

The marginal effect of salinity of up to 5 dS/m on seed yield may also be largely be due to the fact that sandy soils do not retain salts in the manner that clayey soils do. Our results support this assumption, as it was seen that the soil salinity values were on par with those of the irrigation water even after 6 months of irrigation with saline water (Table 1.1). In contrast, salts corresponding to 1 dS/m could accumulate within 3–7 days in more loamy soils [21].

In the control treatment average seed oil percentage by weight (of oil extracted) was determined to be in the range of 23–48 % (Fig. 1.3). According to literature castor seeds generally contain up to 48 % oil of which 42 % can be extracted [22]. The analysis of seed oil content in other treatments is underway, but preliminary results suggest that the oil content is not affected by salinity.

1.4 Conclusions

The results of our study suggest that castor is a suitable candidate for cultivation as a biodiesel feedstock crop in the UAE and in countries with similar climatic conditions. The yield obtained in this study upon irrigation with water of salinity

5 dS/m is on par with the global average [23]. This supports the possibility of cultivating castor using low-moderately saline groundwater and even recycled wastewater. Since castor is not a food-crop none of the associated food versus fuel conflicts apply to its potential as a biodiesel feedstock crop.

Currently, extraction and analysis of oil from the field trials is in progress. In-depth analysis of the systemic and genetic mechanism of salinity tolerance/ sensitivity of castor is also underway. Plant samples are being analyzed to determine the concentrations of various salts in different plant tissues in relation to that of the water and soil in order to understand the specific mechanisms involved in castor's response to salinity. The quantity and quality of oil obtained, efficiency of conversion to biodiesel and fuel performance will also be analyzed in order to confirm that castor seed oil is an economically and qualitatively viable feedstock for biodiesel production in saline environments.

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